

Size-related mortality due to gnathiid isopod micropredation correlates with settlement size in coral reef fishes

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Received: 14 March 2016 / Accepted: 28 December 2016
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Abstract The transition between the planktonic and the benthic habitat is a critical period for the larvae of many demersal marine organisms. Understanding the potential constraints on the timing of this habitat transition, called settlement, is important to understanding their biology. Size-specific mortality can set the limits on lifestyle and help explain ontogenetic habitat shifts. We examined whether size-based mortality risks after settlement may include micropredation by ectoparasites by testing whether survival of settlement-stage fish varies with fish size when exposed to a reef-associated micropredator. Fish (14 species) were exposed to one blood-sucking gnathiid isopod overnight, with appropriate controls; gnathiid feeding success and survival, and fish mortality were recorded relative to fish size. After adjusting for fish relatedness, we found the relationship between fish mortality and size differed with gnathiid exposure: for gnathiid-exposed fish, the mean mortality of the smallest fish was much higher

(57%) than unexposed controls (10%), and decreased to ~0% for fish >12 mm standard length (SL); mortality was almost nil in controls. Thus, a predicted optimal size to switch habitat and reduce mortality risk from micropredation should be >12 mm SL. We then asked what species might be at greater risk and if the steep increase in survival at ~12 mm SL might coincide with settlement at larger sizes among fishes. Across 102 other species (32 families), 61% settled at ≥ 12 mm SL. After adjusting for relatedness, mean fish settlement size was 15.0 mm and this was not significantly different from 12 mm. Thus, settlement size clusters around the minimum fish size threshold our gnathiid experiment predicted would be large enough to survive a gnathiid encounter. These results suggest micropredators may contribute to size-selective mortality during settlement processes and are consistent with the hypothesis that the pelagic phase provides fish an escape from certain micropredators.

Communicated by Ecology Editor Dr. Michael Berumen

Electronic supplementary material The online version of this article (doi:[10.1007/s00338-016-1537-6](https://doi.org/10.1007/s00338-016-1537-6)) contains supplementary material, which is available to authorized users.

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Keywords Ectoparasites · Size-selective mortality · Fish settlement processes · Gnathiidae

Introduction

Complex life cycles are ubiquitous in nature, and thus, understanding their ecology and evolution is an important aim in biology. Changes in size during development cause scaling issues between an organism's morphology and environmental processes (Werner 1988). In fish, adopting a complex life cycle likely addresses this and commonly includes size-dependent ontogenetic changes in behaviour (resource or habitat use). Why such changes might occur gives insight into what might influence the evolution of such traits. Size-specific mortality sets the limits on life

style and can help explain ontogenetic shifts. Werner (1988) argued that ecological constraints on performance based on size may affect ontogenetic size limits of a given body design or life style for a specific ecological situation. Mortality, e.g. predation risk, which can differ among habitats, may also scale with size. Thus, an optimal size to switch habitat might be influenced by mortality risk (Werner 1988).

The offspring of almost all coral reef fishes are released into the surrounding waters where they develop as planktonic larvae prior to returning to the adult demersal habitat (Leis and McCormick 2002). During this transition to juvenile habitat, termed “settlement”, fish encounter many risks. Settlement should therefore occur when larvae achieve a size where their survival would be higher if they settled than if they remained in the pelagic environment. Potential explanations for what could set this minimum size include the relationship between size and developmental competency, increased predation risk in the pelagic environment due to increased vulnerability to a greater diversity of potential predators, and size-based risk of mortality in juveniles after settlement (Miller et al. 1988; Pepin 1991, 2015; Sogard 1997; Holmes and McCormick 2006).

Early post-settlement mortality can be extremely high and is important because juvenile number can regulate adult populations and, if selective, can disproportionately affect body attributes and life history traits (Holmes and McCormick 2006). Sources of mortality include predators, competitors, and parasites. Mortality during the early life of fishes is often size-dependent (Pepin 1991; Sogard 1997; Perez and Munch 2010) and tends to decrease with increasing size (Anderson 1988; but see Blanckenhorn 2000).

Size-selective mortality can also shift with ontogeny, and so, it is important to test the direction of mortality, particularly at critical ontogenetic stages. In Ambon damselfish, *Pomacentrus amboinensis*, a smaller size is favoured at hatching as they have more energy reserves, whereas when transitioning to the benthos faster growth increases fish survival from predators (Gagliano and McCormick 2007; Gagliano et al. 2007). Fish size-selective mortality from predators is often assumed to be due to gape limitation of predators, behavioural selection by the predator, or variation in escape capability depending on prey size (Sogard 1997). In fish settlement processes, the role of predation and competition is comparatively well understood (Holbrook and Schmitt 2002), whereas the role of parasites is not (Cribb et al. 2000; Peyrusse et al. 2012; Sun et al. 2012). Yet fish parasites are universally present on reefs and can affect populations and communities strongly (Adlard and Lester 1994; Forrester and Finley 2006). Here, we examine an even less-known group of

parasites, the micropredators, but one that is exceedingly common on coral reefs, and how they affect fish at an important life stage.

Parasites and predators are often overlapping classes of natural enemies (Raffel et al. 2008). Micropredators are considered different from parasites in that they attack several hosts like a predator, but take only a small meal like a parasite, often feeding on blood (Kuris and Lafferty 2000; Lafferty and Kuris 2002). While micropredation is often brief, it can still greatly affect host populations (Edman and Scott 1987). Theory suggests that micropredators will impact small hosts more than large ones (Kuris and Lafferty 2000; Lafferty and Kuris 2002). Marine examples include leeches (Kuris and Lafferty 2000) and caligid copepods (Kuris 1997); gnathiid isopods are the most common micropredators on coral reefs (Grutter and Poulin 1998; Grutter 2008). Gnathiids feed only during their three juvenile stages, engorging on fish blood before returning to the reef to digest their meal and moult to the next stage (Smit and Davies 2004). Pathological sequelae are evident in aquaria, fisheries, and in nature (Smit and Davies 2004). Gnathiids are central in fish cleaning behaviour; many cleaner fishes eat them (Grutter 2002) and subsequently reduce their loads on fish (Grutter 1999).

Gnathiids naturally occur on juvenile fishes (Penfold et al. 2008; Grutter et al. 2011). Hence, they may be a cause of morbidity and mortality for small, young fish. Attack by one individual reduces successful settlement in *P. amboinensis* (Grutter et al. 2011). Repeated attacks reduce juvenile growth (Jones and Grutter 2008). Gnathiids kill some juveniles, usually when more than one gnathiid or a small host (<10 mm standard length, SL) is involved (Jones and Grutter 2008; Penfold et al. 2008; Artim et al. 2015). Gnathiids occupy most reef benthic microhabitats (Jones and Grutter 2007). Importantly, their small size (Grutter 2003, 2008) should make them difficult for even small fish to avoid. Escaping the sublethal, or even lethal, effects of micropredators like gnathiids would surely be an advantage for very small fish. This study thus uses gnathiid isopods as a model micropredator to examine the new hypothesis that size-based mortality risks after settlement include micropredation.

Therefore, to determine whether micropredators are a potential source of mortality and thus act as selective agents for fish size at settlement, we tested whether gnathiids attack very small fish immediately prior to and at settlement and whether there is a minimum size threshold at which fish survive a gnathiid attack. We then compared how this minimum size corresponds to the size of fish at settlement across many taxa to reveal which species might be vulnerable to gnathiids and also whether their overall size distribution suggests selection for a particular size. We exposed individual juvenile fish to a single gnathiid

overnight in the laboratory and examined the outcomes for both participants in relation to fish size. We then asked if the steep increase in survival at the predicted minimum SL threshold might act as a general constraint to settlement at smaller sizes among coral reef fishes by comparing the mean size of fishes with the size threshold obtained from the experiment.

Materials and methods

Study species and fish collection

Some fish taxa can be collected just before settlement with light traps (Meekan et al. 2001), and *Gnathia aureusmaculosa* gnathiids can be cultured (Grutter 2001, 2003) and are common on adult fishes at the study site (*Gnathia* sp. A in Smit et al. 2006). This combination makes an ideal model system for testing juvenile fish and gnathiid interactions. Fish were collected at Lizard Island, Great Barrier Reef (14°40'44.17"S, 145°26'53.90"E), in light traps moored overnight off the reef edge (~500 m from shore). Gnathiids were from an existing culture maintained at Lizard Island Research Station. The third developmental stage was used because its larger size (mean \pm SE, unfed: 1.1 \pm 0.01 mm; Grutter 2003) made it easier to work with. This size occurs on wild juvenile fish and is not correlated with fish SL (Penfold et al. 2008; Grutter et al. 2011).

Laboratory experiment

Between 14 November and 22 December 2006, fish (total $n = 466$) were transferred from light traps (~0700 h) to aquaria (65 \times 35 \times 30 cm) with aeration and water flow. Fourteen different types of fishes were used, representing four families plus two types which could not be identified to any taxonomic level. Fish type was selected based on abundance. Most fishes were settlement-stage individuals with SL ranging from 7.0 to 19.7 mm. However, two fishes (Apogonidae2: 6.1–7.7 mm SL; Pomacentridae3: 7.9–9.9 mm SL) were identified as late-stage larvae (i.e. “pre-settlers”) based on their limited juvenile body colouration and were included to extend the range of small-sized fish. The effects of gnathiids on hosts likely depend on the amount of blood removed relative to the total blood volume of the host. Blood volume is likely more a function of body mass than length. While body mass tends to show a strong relationship with length within species and thus is a good proxy measure for mass, the relationship between length and mass can vary among species according to their shape (Jobling 2002). Thus, mass may have been a more reasonable measure to use than length. However, since

surviving fish were returned alive to the wild, we used the less stressful body measurement of length.

Unfed gnathiids were collected from the culture tank with a pipette on the test day (same day fish were removed from light traps) and added to half (randomly selected) of the test plastic containers (16 \times 11 \times 4 cm, 365 mL seawater). Around 1900 h, one fish was randomly assigned to a treatment (one or no gnathiid). The following morning (1030 h), fish survival and SL and the status of the gnathiid (fed, unfed, or gnathiid missing—presumably eaten by the fish) were recorded. Fish were measured in sea water in a plastic bag and returned to the reef. Sample sizes were influenced by fish and gnathiid availability. We examined the probability of an event occurring in relation to fish size, adjusted for fish relatedness, separately for the three parameters measured (see statistical analyses). Since micropredation by a gnathiid and predation by the fish are mutually exclusive in juveniles (Penfold et al. 2008), we assumed the same here.

Size of fishes at settlement

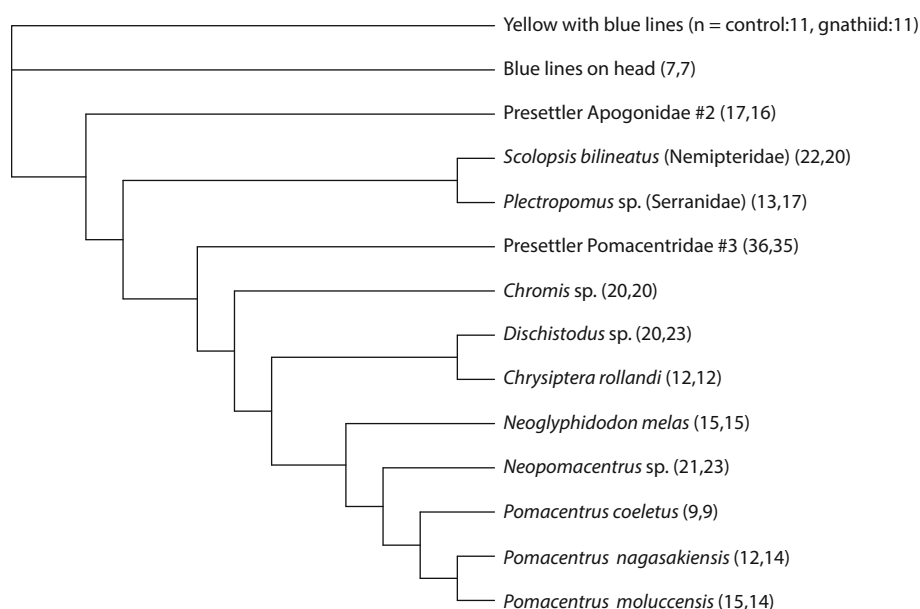
We obtained fish SL at settlement from 102 fish species (32 families, 6 orders, total $n > 2261$ individuals, see electronic supplementary material, ESM Methods, Table S1 for details). We largely used data from Lizard Island to reduce potential spatial variation.

To examine the potential risk of mortality of individual species, we plotted the mean SL of each fish type arranged according to fish phylogenetic relatedness with a tree and compared their SL to the predicted minimum SL threshold at which fish are expected to survive a gnathiid isopod encounter (ESM Fig. S1a, b). We then tested whether the overall mean size of all fishes, adjusted for relatedness, differed from the predicted minimum SL threshold obtained in the experiment (see statistical analyses).

Statistical analyses

The software R v 3.1.0 (R Core Team 2015) was used in all statistical analyses, except where stated. For the experiment, a phylogenetic supertree of fish types was constructed from several trees (Helfman et al. 1997; Pomacentridae: Frederich et al. 2013; Near et al. 2013) according to the family, genus, or species used in our experiment (Fig. 1). A logistic regression approach was used to determine, separately, whether fish SL was a significant predictor of whether a fish had died, a gnathiid had fed successfully, or the gnathiid had been eaten. We used Grafen branch lengths (Grafen 1989) because, having several trees, we could not use time as the unit for branch length. This method assigns a height to each node one less

Fig. 1 Phylogenetic supertree of fish types (n = not exposed or exposed to one gnathiid) used in the experiment



than the number of species below or at that node; the length of each path segment is then the difference between the height of the upper and lower nodes. This has the advantage of treating taxonomic ranks as arbitrary.

We used JAGS software (Plummer 2012), which performs Bayesian analyses with Markov chain Monte Carlo. We calculated the predicted mortality and the 95% central posterior density interval (i.e. credible interval or CrI, equivalent to a confidence interval; strong relationships are ones where the 95% CrI does not encompass zero) for a given SL. To determine the odds (or probabilities) of settlers dying, the data were classified as alive (0) or dead (1) and fish SL was treated as a continuous covariate. The treatments tested were no gnathiid (control) or exposure to one gnathiid, regardless of the feeding success of the gnathiid. This latter a priori decision was made because we assumed that even if the gnathiid had not fed, the fish still could have experienced stress from its presence alone, or its attempts to feed; gnathiids have been observed attempting to feed at more than one site prior to selecting a final feeding site and so likely wounding the fish even if they had not completed feeding (ASG, pers. obs.).

We reported our analysis in accordance with guidelines for the reporting of Bayesian analyses in clinical studies (ROBUST) criteria (Sung et al. 2005). The outcomes estimated were the posterior distributions of the slope and intercept for the relationship between SL and the probability of fish mortality (or gnathiid feeding success or gnathiid eaten) on the logit scale (a logit link function was used). We used vague Gaussian priors for both the intercept and slope [i.e. $\text{Normal}(0, 10^7)$]. Since the study was performed on multiple species or types, we wished to incorporate phylogenetic information into our analysis, as the

values for individual species may not be independent of each other. That is, closely related species may be more likely to have similar mortality curves than more distantly related species. To do this, we included a random effect for species, with prior $\text{Normal}(0, \Sigma)$, where Σ is the phylogenetic correlation matrix derived from the tree (Fig. 1). We used Grafen's branch lengths with $\rho = 1$ (Grafen 1989). For each outcome, we ran three chains. For each chain, we discarded the first 10,000 iterations as "burn-in". We then performed a further five million iterations with a thinning interval of 500. Convergence was assessed by eye and also with the convergence diagnostics in the "coda" package (Plummer et al. 2006).

For the size at settlement data, we created a supertree for the majority of species, using Near et al.'s (2013) acanthomorph phylogeny as the backbone at the family level, and augmented any missing species with other available trees [Helfman et al. (1997), for some families, certain trees were used—Chaetodontidae and Labridae: Cowman and Bellwood (2011), *Cheilinus* sp. and *Coris schroederi* (both Labridae) were placed in the same branch as the majority of other species in that genus; Pomacentridae: Frederich et al. (2013), species whose phylogeny was not available in the literature were placed in the family following Near et al. (2013), *Cephalopholis* sp. was placed in the Epinephalidae following Near et al. (2013), whereas Serranidae as the family were retained for all the other so-called serranids]. Some genera were assigned to a specific branch if they were the only one of that genus.

We estimated the predicted size of fish at settlement, relative to the ancestral size, with the phylogenetically independent contrast "pic" method in the ancestral character estimation "ace" function of the "ape" (Paradis

2012); Grafen's branch lengths with $\rho = 1$ (Grafen 1989) were employed. To determine whether the overall mean size of all other fish at settlement differed from the predicted minimum SL threshold, we calculated the overall phylogenetically adjusted mean SL for 102 species; we used a generalised least squares fit by restricted maximum likelihood with the correlation structure "corPagel" in the "ape" package (Paradis 2012). We calculated Pagel's λ and Blomberg's K with the "ape" and "picante" packages, respectively (Kembel et al. 2010). Tests of hypotheses for $\lambda = 0$ or $\lambda = 1$ and $K = 0$ were also conducted. Because the data were skewed towards larger individuals, we normalised the data using a natural log transformation. The adjusted mean was then tested against the log of the hypothesised minimum SL threshold with a one-sample t test.

Since SL at settlement was available only for a subset of the species per family that occur at Lizard Island, its distribution per family could be biased if some families' species numbers are underrepresented. Therefore, we determined all the species at Lizard Island per family with species records and calculated the percentage sampled (ESM Methods; ESM Table S2).

Results

Laboratory experiment

Fish mortality

Overall, regardless of fish type or SL, 2% of fish (0–33% per fish type) died in the control treatment. In the gnathiid treatment, 11% of fish (0–49% per fish type) died; the highest mortalities were in those fish identified as pre-settlers (Apogonidae2: 38%, Pomacentridae3: 49%) with the other mortalities occurring in *Scolopsis* sp. (5%), *Neoglyphidodon melas* (7%), and *P. nagasakiensis* (7%) (ESM Table S3). The overall mortality of fish differed according to whether they were found with fed (17.7%), not fed (4.1%, involving fish of 6.1, 6.3, and 12.5 mm SL), and presumed eaten (2.6% involving a fish 6.8 mm SL) gnathiids (Likelihood ratio $\chi^2_1 = 13.4$, $P = 0.0012$, JMP 9.0).

After controlling for fish relatedness, there was a strong interaction between exposure to gnathiid treatment and fish SL on the probability of settlers dying. The Bayesian analysis parameter estimate for gnathiid exposure x SL interaction term was -1.2914 (95% CrI = -2.120 to -0.6071 ; ESM Table S4; Fig. 2). When predicted values in relation to SL were plotted separately for each parasite treatment, mortality in settling fishes exposed to a gnathiid was higher for smaller fish. For example, for exposed fish

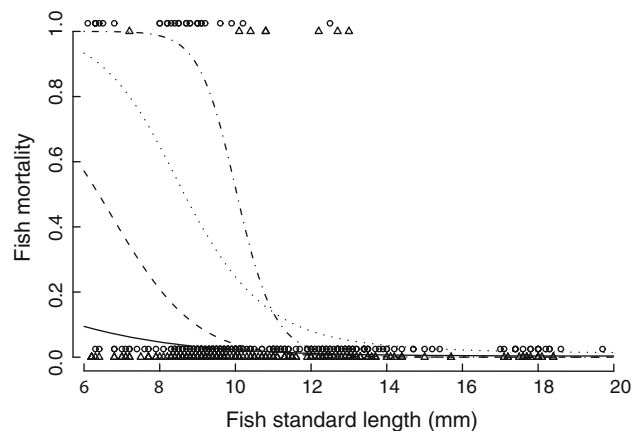


Fig. 2 Fish mortality in relation to fish standard length; symbols represent dead (1) or alive (0) fish for fish exposed to a gnathiid isopod (circle) or not (triangle); symbols are jittered. Predicted values and 95% central posterior density interval (credible interval or CrI) are represented by lines (gnathiid-exposed: dashed or dash-dotted; no gnathiid: solid or dotted)

6 mm SL the estimated average probability for mortality (estimated error: 95% CrI) was 57% (0–100%), compared to 10% (0–100%) in fish greater than 12 mm SL.

Gnathiid feeding success

Gnathiids were attached at various sites, including pectoral and caudal fins, opercula and eyes (Fig. 3). Gnathiids successfully fed on the fish in 52% of trials (range: 11–76% per fish type; ESM Table S3); after controlling for fish relatedness, the probability of feeding was not strongly correlated with fish SL [Bayesian analysis parameter estimate = 0.04455 (95% CrI = -0.1132 to 0.1867); ESM Table S4; ESM Fig. S3]. Gnathiids fed on some of the smallest and the largest fish tested.

Gnathiid predation by fish

The gnathiid was lost and presumed eaten by the fish in 16% of trials (range: 0–56% per fish type; ESM Table S3). After controlling for fish relatedness, the probability that a gnathiid was eaten was not strongly correlated with fish SL [Bayesian analysis parameter estimate = 0.04862 (95% CrI = -0.19192 to 0.2471); ESM Table S4; ESM Fig. S2]. Gnathiids were eaten by some of the smallest and largest fish tested.

Size of fish at settlement

The size of fish at settlement varied greatly; its distribution was skewed towards larger fish, and the median size was 13.0 mm SL (Figs. 4, 5; ESM Fig. S1a, b). The overall distribution revealed a relatively narrow size range at



Fig. 3 Gnathiid isopod *Gnathia aureusmaculosa* feeding on *Pomacentrus moluccensis* settler

settlement, with 50% of species settling between 9 and 15 mm SL. Sixty-one species (60%), belonging to 88% of families, settled at a SL \geq 12 mm (12.0–185.5 mm), the minimum size our experiment predicted would be large enough to survive a gnathiid encounter. These species belonged to 28 families (Fig. 5). In contrast, 41 species (40%), belonging to 25% of families (some families had species <12 and >12 mm), settled at a SL < 12 mm (minimum 2.87 mm) and so are predicted to be at risk of dying from a gnathiid encounter; these belonged to eight families: Apogonidae, Callionymidae, Chaetodontidae, Gobidae, Labridae, Plesiopidae, Pomacentridae, and Scorpaenidae (Fig. 5).

The phylogenetic tree of the 102 species was relatively consistent with other recent phylogenies (Betancur-R et al. 2013; Riginos et al. 2014). Pagel's λ (0.6692) was

significantly different from zero and one (both $P < 0.0001$), indicating a phylogenetic signal; Blomberg's K (0.0553) was much less than one, yet significantly different from zero ($P = 0.009$), implying that relatives resemble each other less than expected under Brownian motion evolution along the specified tree (Blomberg et al. 2003). The overall phylogenetically adjusted mean log SL (mean $-$ SE, mean $+$ SE.) when back-transformed was 15.0 (11.9, 18.7) mm, which was not significantly different from the log predicted minimum size threshold of 12 mm ($t = 0.9869$, $df = 101$, $P = 0.3026$). The predicted size of fish at settlement, relative to the ancestral size at settlement, also varied greatly; some families had species with a wide range in predicted sizes (e.g. Pomacentridae), whereas some families had mostly small fish (e.g. Labridae) or very large fish (e.g. Monacanthidae; ESM Fig. S1a, b). The mean (SE) per cent of species sampled per total recorded for each family at Lizard Island was 20 (3)%; the 32 families sampled involved 36% of all the families recorded there (Fig. 5; ESM Table S2b).

Discussion

We tested the hypothesis that demersal micropredatory parasites cause size-selective mortality and thus might be a potential-selective agent for the minimum size at which fish settle onto the reef. Experimentally, we showed there is a minimum size (12 mm SL) at which fish survived a micropredator attack. When compared with the mean size at settlement per species for 102 fishes, 40% of species settled at a size <12 mm SL and so are predicted to be at risk of dying from a micropredator encounter based on size

Fig. 4 Mean fish standard length at settlement presented as **a** frequency per size class of fish in 3 mm increments (for simplicity, only every second size class is labelled for fish <33 mm) and **b** boxplot; median is enclosed by *box* representing the interquartile range, *error bars* represent the 10th and 90th quantiles, and *filled circles* are outliers. The *dashed line* represents the predicted minimum size threshold (12 mm) at which fish are expected to survive a gnathiid isopod encounter

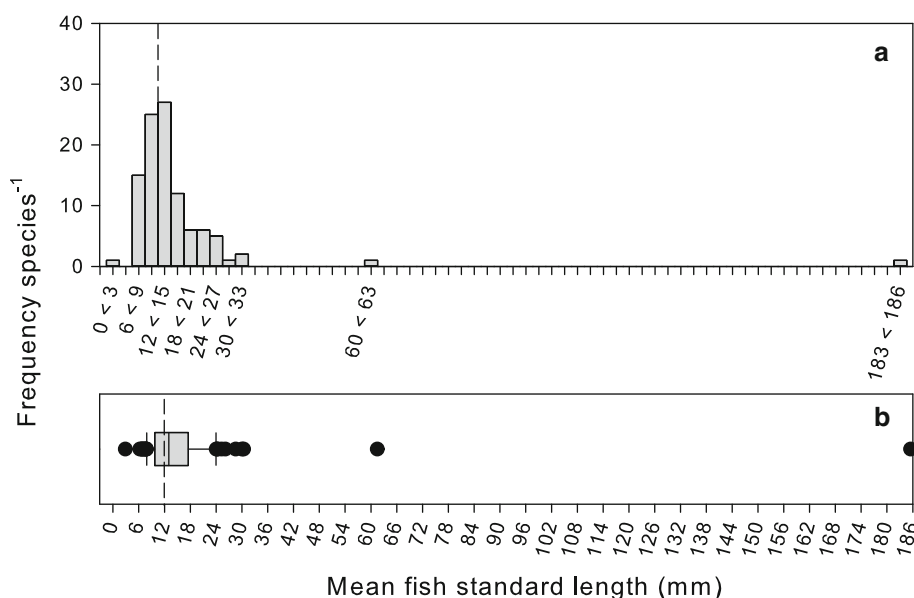
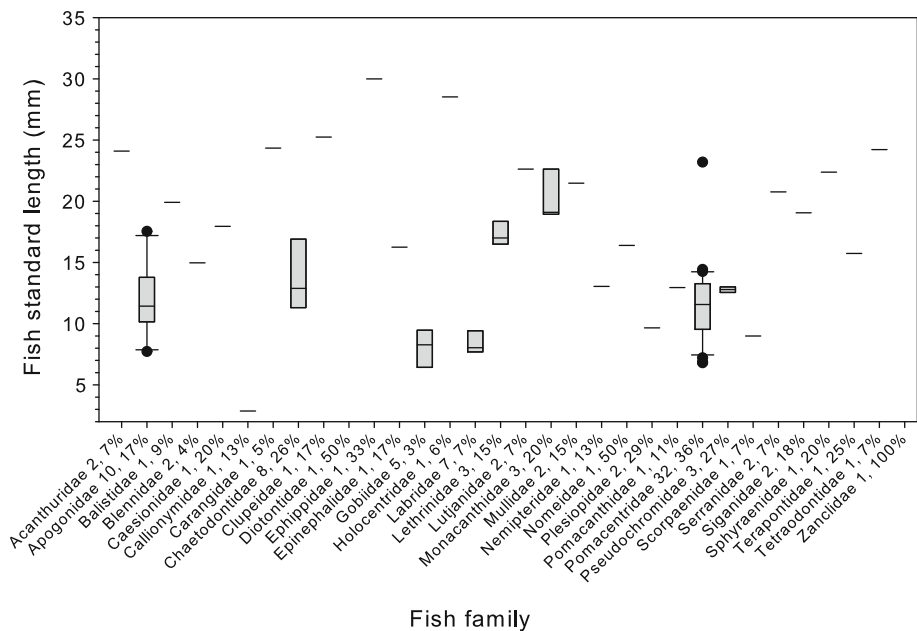


Fig. 5 Boxplots of fish standard length at settlement per family. Each family is followed by number of species sampled and the per cent of all species recorded per family at Lizard Island. See Fig. 4 for *dashed line* and boxplot definitions (*lines without boxes* are medians). Zanclidae (61.5 mm) and Diodontidae (185.5 mm) were excluded for ease of interpretation



alone. Intriguingly, the minimum survival size obtained from the experiment and the overall phylogenetically adjusted mean SL of the 102 species coincide with each other. This correlation suggests that micropredators may be one of the multiple factors that contribute to selection of size at settlement. Furthermore, the pelagic phase may afford fish protection from these reef-based parasites when fish are very small.

Laboratory experiment

The curves of fish mortality and size differed depending on whether or not fishes were exposed to a gnathiid isopod micropredator; gnathiid-exposed fish showed a dramatic decline in mortality with fish size. For the smallest gnathiid-exposed fish (6 mm SL), an average 57% died; but this rate dropped rapidly to almost 0% in 12-mm-SL fish (Fig. 2). The estimated error (95% CrI) for this mortality was very high (0–100%), but did not extend much past 12 mm SL, emphasising the lack of mortality in the larger fish; in contrast, mortality was almost nil in controls. A similar rate of mortality with size occurs within juvenile *Acanthochromis polyacanthus*, where mortality decreases to nearly zero in fish greater than ~12 mm SL (Penfold et al. 2008). However, *A. polyacanthus* does not have a pelagic larval phase (Leis and McCormick 2002). Similarly, Adlard and Lester (1994) found the cymothoid isopod *Anilocra pomacentri* caused greater mortality in small juvenile *Chromis nitida* compared with larger individuals. Assuming our fish species had a similar length–weight relationship to *Acanthochromis polyacanthus* (Penfold et al. 2008), the exponential increase in mass with length

could explain the rapid decline in mortality in our curve since blood generally increases in proportion to body mass. Thus, gnathiids would remove proportionally less blood with increasing fish size. Size-biased mortality in larval fish has largely been attributed to the behavioural selection or gape limitation of predators, or variation in escape capability with prey size (Sogard 1997). This study suggests gnathiid isopod micropredation is an additional source of size-based mortality.

The highest mortalities (Apogonidae2: 38%, Pomacentridae3: 49%) were in the stage prior to the developmental stage known as “competence”, which enables fish to leave the water column and join the demersal population (McCormick 1999). These pre-settlers were some of the smallest fishes tested, with a SL (mean \pm SE) of 6.8 ± 0.1 and 8.8 ± 0.1 mm, respectively. The size of other species (*Chromis* sp., *Dischistodus* sp., *Neoglyphidodon melas*, and *Scolopsis* sp.) also overlapped with that of pre-settlers (ESM Table S3). Whether size alone or a factor related to development affected mortality remains unclear. For example, fish undergo dramatic shifts in their body dimensions, physiology, eye structure, level of tissue maturation in skin, and behaviour during metamorphosis from larvae to juveniles (McCormick and Makey 1997; McCormick et al. 2002; Power et al. 2008). However, Penfold et al. (2008) also found a similar relationship of mortality with SL in a species that does not have a dramatic metamorphosis, suggesting that SL (or a correlate) may be more important than developmental stage.

The size of the fish relative to the micropredator (Fig. 3) is likely to make smaller fish highly vulnerable to blood loss. Gnathiids feed on fish blood, piercing the fish with

their mouth parts and sucking blood until they become engorged (Smit and Davies 2004), a process that can take several hours in relatively small individuals (Grutter et al. 2011; Penfold et al. 2008). Gut volume of an engorged third-stage *G. aureamaculosa* is 2 mm³ (Grutter 2003). For a settlement-stage *P. amboinensis* (11.5 ± 0.1 mm SL), this is estimated as 85% of their total blood volume (Grutter et al. 2011). The fish tested here included individuals almost half this size, and thus, the likely impact of the gnathiid would be even greater. The loss of so much blood is presumably highly detrimental, both from the wound and its removal by the gnathiid. The largest juvenile stage of gnathiid (engorged: 1.5–2 mm; Grutter 2003) was used, but this probably overestimated the pathogenicity of a typical infection. For comparison, gnathiids 0.8–1.4 mm were found on wild fishes (Penfold et al. 2008; Grutter et al. 2011). The higher mortality associated with fed (17.7%) compared with unfed (4.1%) gnathiids suggests mortality was largely due to the feeding activities of gnathiids and that the a priori decision to include unfed ones in the analysis, due to their potential effect on fish via stress, was a conservative one. The ratio of predator size to prey size has been used to explore predator–prey interactions where the predator is generally much larger than the prey (Miller et al. 1988), but this study shows that much smaller ratios of micropredator to prey sizes can also cause mortality. We used length as a measure of body size. However, as blood volume is likely more closely related to fish mass than length, mass would have been a more reasonable measure of body size. For example, there was likely variation in the mass of some same-sized fishes, such as in the 9–10 mm range that included a shallow-bodied nemipterid among the deep-bodied pomacentrids (ESM Table S3). Thus, this additional variation due to body mass was not accounted for. Whether using mass would have affected the outcome is not likely, however, since 65% of the fishes were pomacentrids with similarly shaped bodies and they spanned almost the entire size range of fish tested (8.8–14.9 mm).

While only 3.5% of wild *P. amboinensis* juveniles had a gnathiid when sampled at dawn, the relatively short attachment period of gnathiids on fish suggests that over several days the cumulative infestation rate on individuals would be higher at this time of day (Grutter et al. 2011). Furthermore, although a single gnathiid did not directly kill *P. amboinensis* settlers, their performance and successful establishment on the reef were reduced (Grutter et al. 2011). The negative effects of gnathiids likely also increase the interactions of infected settlers with predators and thus may further contribute to the high mortality observed at this time.

Gnathiid feeding success was not correlated with fish SL, a pattern also observed in *Acanthochromis*

polyacanthus (Penfold et al. 2008). Thus, our higher mortality in smaller gnathiid-exposed fish was not the result of greater gnathiid feeding success. Seventeen per cent of gnathiids disappeared, presumably eaten by the fish, and this mortality was also not correlated with fish SL. Since fish as small as 6.8 mm SL ate a gnathiid, fish gape width was unlikely a limiting factor. In contrast, the probability of a gnathiid being eaten increased with fish SL for *A. polyacanthus* (Penfold et al. 2008). However, being entirely benthic, larger *A. polyacanthus* would have more experience eating benthic prey than smaller, younger fish. Our light-trapped fish presumably only had experience with feeding on pelagic organisms.

Size of fish at settlement

The overall median size of species at settlement was 13.0 mm SL. A frequency distribution and boxplot (Fig. 4) of settlement size showed the data were skewed towards larger fish indicating a tendency for species to settle at ≥12 mm SL; intriguingly, also a minimum size threshold our gnathiid experiment predicted would be large enough to survive a gnathiid encounter. Similarly, across families, a large proportion (88%) of families also settled at ≥12 mm SL. This skew suggests selection favours fish greater than 12 mm SL at settlement. Body size during larval and juvenile stages is an important determinant of survival. Size-selective mortality is often biased against smaller individuals (Sogard 1997). Larger individuals are presumed to be better able to escape from predators, endure physiological extremes, avoid starvation, compete, and acquire shelter compared to smaller conspecifics (Sogard 1997; Holbrook and Schmitt 2002; Holmes and McCormick 2009).

When size was controlled for potential phylogenetic (relatedness) influences, the overall mean settlement SL was 15.0 mm. This size was not significantly different from the predicted minimum size threshold of 12 mm, indicating there was clustering around 12 mm. Forty per cent of species from eight families settled at SL < 12 mm and so, all things being equal, are predicted to be at higher risk of mortality from a gnathiid encounter. This variation among species, however, may be the result of species-specific size responses to the same selective pressure or different selective pressures driving the evolution of different species. Indeed, some of these other pressures such as predation, food and shelter acquisition, competition, and the physical environment (Sogard 1997) may simply be more important than gnathiid predation risk.

Clearly, there are still some very small settling fishes. Therefore, the relative risk posed by gnathiid micropredators may not always be an important factor. Smaller juveniles may benefit from a reduction in the costs of being

large, such as viability costs due to predation, parasitism, and starvation from reduced agility, increased detectability and higher energy requirements and from longer development and/or fast growth (Blanckenhorn 2000).

The size of fish at settlement showed a narrow peak around 12 mm SL, with 50% of species ranging between 9 and 15 mm SL. Size at settlement is a balance between processes/agents that encourage a fish to undergo metamorphosis and those that would discourage some fish to undergo metamorphosis when too small. Thus, here gnathiid isopods appear to be one of the agents that would discourage fish to undergo metamorphosis before achieving a minimum size (~12 mm SL). In other vertebrates with similarly complex life cycle, such as anurans, the tadpole's size at metamorphosis is influenced by its tail, which is without vertebrae for rapid elimination (Wassersug 1989). This rapid metamorphosis was likely selected due to high predation at this life stage (Arnold and Wassersug 1978). The maintenance of the tadpole stage may be due to size-specific mortality (Werner 1988).

While size at metamorphosis is generally tightly constrained within a fish species, there are some exceptions (e.g. some wrasse and surgeonfishes; Cowen 1991; McCormick 1999). However, these tend to be fishes that have a well-developed ability to delay metamorphosis (Leis and McCormick 2002), suggesting there are taxon-specific trade-offs associated with size at settlement. If large size is so important for surviving gnathiid infection, then why aren't all new settlers huge? Numerous ecological, physiological, and evolutionary costs of being a large organism have been proposed (Blanckenhorn 2000). While the likelihood of parasitism may be an important factor constraining the variability in size towards larger size classes, the exact trade-off will depend partly on the ecology of the individual species and how this ecology changes during the settlement transition.

Many factors, however, are correlated with size of fish at settlement. Age (pelagic larval duration) is positively correlated with size within and across species (Brothers and Thresher 1985; Soeparno et al. 2012). Interestingly, when variability in the size at settlement has been compared with variability in age at settlement, there is always more variation in the latter (McCormick 1999). This suggests that size at settlement is not simply a direct result of time spent in the plankton. Also, maximum burst and swimming speed and swimming endurance increase with larval fish size (Bailey 1984; Leis et al. 2013). These traits could also influence interactions with gnathiids if older fish are more experienced with avoiding harmful organisms or their morphology or physiology is more developed, making them less vulnerable.

Avoiding gnathiid-rich habitats should benefit smaller juveniles the most. Gnathiids occur in macroalgae

(Cacabelos et al. 2010), polychete tubes, or sponges (Ota et al. 2008; Tanaka and Nish 2008). However, some scleractinian corals repel or kill gnathiids (Artim and Sikkell 2013). Interestingly, many fishes use such corals as a nursery habitat (Anderson et al. 2007). Whether gnathiids influence fish habitat choice, however, remains unknown.

The species used here are not a random sample of the population, and therefore, the conclusions are biased towards the mix of species for which data exist. Light traps are very selective: many smaller species rarely enter them and fish abundance and diversity are variable (Choat et al. 1993). The experiment was dominated by damselfishes (Pomacentridae, 64%). For the settlement-size data set, additional species from other sources were included to increase the breadth of families analysed. On average, only 20% of species per family were sampled and the families involved only 36% of all the families recorded at Lizard Island (ESM Table S2). Nevertheless, the actual sizes for all sampled species relative to the predicted minimum size threshold of 12 mm are provided (ESM Fig. S1a, b). Such information will provide a foundation for other studies to further explore the questions raised here.

Our results are consistent with the hypothesis that a pelagic phase provides fish an escape from demersal parasites (Combes 2001; Strathmann et al. 2002). Pelagic life has been suggested as a means to avoid local transmission dynamics of parasites, preventing transmission from parents to offspring, or to lower transmission rates generally, because densities are much lower in the open ocean away from the reef. In fish, some individuals do acquire parasites largely during or after settlement (Grutter et al. 2010; Peyrusse et al. 2012), but others acquire them in the pelagic environment (Cribb et al. 2000; Sun et al. 2012). This study and other recent work suggests that avoiding reef-based micropredatory parasites may be another compelling benefit of leaving the reef (Grutter et al. 2008, 2011; Jones and Grutter 2008; Artim et al. 2015). Although we did not test very early larval stages of fish, they would be even smaller and so most likely also at high risk of mortality from micropredators; however, minimum host sizes have not yet been determined. The observed variation in the mean size at settlement across taxa and within species, however, suggests that multiple agents likely exert selection on size at settlement. The concordance between the size at which fish are susceptible to micropredators and settlement size suggests gnathiids may be one of these agents of selection, though further studies are required to determine whether they are one of the primary causes of this relationship.

Acknowledgements Thanks to Justine Becker, Angela Crean, Conor Jones, Lynda Curtis, Rachel Fogelman, Gay Marsden, Sarah Pausina, and Carlos Vargas for field assistance, Lizard Island Research Station staff and the many volunteers who maintained the gnathiid culture, and Peter J Doherty, Jeff M Leis, Cynthia Riginos, and anonymous

reviewers for comments on the manuscript. This research was funded by the Australian Research Council and The University of Queensland. Peter J Doherty provided some of the size data. Anne K Hoggett created the Lizard Island species list.

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